Evaluation of antifungal potential of synthesized metal complexes against *Trichophyton tonsurans* using molecular docking approach

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ABSTRACT
In the past decades, the incidence of opportunistic fungal infections has increased tremendously and the spectrum of fungal pathogens has changed. It is therefore important to investigate new metal complexes that could be used to treat dermatophytoses. Standard chemical method was used to synthesize complexes from two metals and *in silico* evaluated against lanosterol 14-α-demethylase of *Trichophy tontonsuran* was done by AutodockVina software. The structure of the synthesized ligand was drawn and stabilized using Marvinskectch version 19.13 and saved in SDF and PDB format while the structure of the *T. Tonsuran* lanosterol 14-α-demethylase was obtained from the protein bank (strain XA67with RefSeq Selected Product:ADI76636.1). The melting points of Cobalt(II)(Trimethoprim)$_2$ chloride complexes ranged range between 208 and 219 °C while their percentage yield ranged between 37 and 61 %. Cadmium (II) (trimethoprim) chloride complex yields between 45 and 64 % with lowest and highest temperature of 200 and 233 °C respectively. The result of the screening shows that $CdL_2H_2OSO_4$.SCN has the highest affinity for the *T. Tonsuran* protein with -11.2 kcal/mol. A result similar to the standard ligand docked into the active site of the protein. Other ligands with close biding affinity to the protein are $CdL_2H_2OSO_4$, CoL_2ClH_2O and CoL_2ClH_2O.SCN with -9.3, -9.3and 8.9 kcal/mol respectively. These synthesized complex metals showed a promising anti-*Trichophyton* potentials.

Key words: Metal complex, lanosterol 14-α-demethylase, *in silico,* dermatophytoses, *Trichophyton*

1. INTRODUCTION

Tineacapitis is a common infection among school-age children especially in the subtropical Africa. Its transmission is through person to person or indirectly through contact contaminated inanimate objects (Gupta and Ramnani, 2006; Bongomin *et al.,* 2017).The incidence of the infection is very common among the poor in rural area and regions where people do not have adequate access to water. Tineacapitis is characterized by spreading, scaly, irregular or well-demarcated areas of erythema and alopecia (Moto *et al.,* 2015). *Trichophyton* species has been reported to be a principal causative agent of tineacapitis though this depends on location, environments, climates, ethnic groups and life styles. The resistance of the organism to antifungals has called for application of biological and chemical products to curtail the growth of the fungus (Kakande *et al.,* 2019).

Metal complexes have been reported to interfere with biological activities. Their activities are largely dependent on their metal ions, ligands and structures. These factors are responsible for their interaction with other molecules and their antimicrobial activity (Cohen, 2007; Lalehzar *et al.,* 2008). The exploration of drug-target recognition and binding from both the mechanistic and energetic points of view to know the relationship between the molecules using virtual screening method or molecular docking is desirable. It aids predictive modelling and also helps to detect potent chemical compound and potential drug candidates for treatment of different infectious diseases.

A method that has the advantage of low cost and efficacy and is ligand and structure based predicts a better drug for treatments. Therefore, the aim of this study is to investigate the interaction of two synthesized metal compounds of cadmium trimethoprim thiocyanate sulphate and cobalt trimethoprim thiocyanate chloride complexes at different mixing ration with fungal protein lanosterol 14α-demethylase.

2. MATERIALS AND METHODS

Reagents for Analyses

All reagents used are of good analytical grades and were used without further purification. Trimetrioprim obtained a gift from the Chinese pharmaceutical company Ibadan, potassium thiocyanate, cadmium chloride and cobalt chloride salts are from May and Baker pharmaceutical company.

Synthesis of Cobalt (II) Trimethoprim chloride complex

The complexes were synthesized by direct mixing method. (0.29 g) 0.005 mol. Trimethoprim (ligand) was dissolved in 10 mL distilled water and (0.24 g) 0.005 mol. Cobalt (II) chloride ($CoCl_2.6H_2O$) were carefully weighed and dissolved in 10ml distilled water separately. 0.005 mol. Sodium carbonate salt was also dissolved in 10 mL distilled water and added to colouless solution of Trimethoprim to deprotonate the ligand. The colourless solution of Cobalt (II) chloride was then added to the mixture, both were stirred continuously for 3 h. The resulting solution was filtered through a sintered glass porosity No: 4 and a lilac residue obtained was kept inside a desiccator containing silica gel for 5 days until a constant weight achieved according to Heba (2004).

The ratio 1:2 Cobalt(II)(Trimethoprim)$_2$ chloride complex was also synthesized, by following the ratio 1:1 procedure but the molar concentration of the ligand increased by 2. i.e. (0.58 g) 0.01 mol. trimethoprim ligand. However, mixed ligand thiocyanate complexes were synthesized in ratios 1:1:1 and 1:2:1 by preparing 0.005 mol (0.059 g) of potassium thiocyanate and dissolved in 10ml distilled water to metal/Trimethoprim mixture but in ratio 1:2:1, the molar concentration of the ligand was doubled. The process of filteration, washing, drying remained the same to obtained light blue and brown colours.

Synthesis of Cadmium (II) (trimethoprim) chloride and its mixed ligand

Direct method was also adopted. (0.29 g) 0.005 mol. Trimethoprim ligand and 0.005 mol. Cadmium (II) chloride were prepared separately and dissolved in 10ml distilled water. The ligand was deprotonated by adding 10 mL (0.005 mol.) Sodium carbonate, to the mixture, a colourless solution of Cadmium (II) chloride was added. The reacting mixture was stirred continuously for three hours and the mixture was filtered through sintered glass porosity No: 4 to obtain a pure residue which was dried inside a desiccator for 5 days. Ratio 1:2 cadmium (trimethoprim) chloride was synthesized by increasing the concentration of the ligand to two .0.58 g (0.1 mol.) Trimethoprim dissolved in 10 mL distilled water. Furthermore, mixed ligand complexes in ratio 1:1:1 and 1:2:1 were also generated by carefully preparing 0.005 mol. (0.0059 g) potassium thiocyanate in distilled water but the molar concentration of the ligand increased by two in ratio 1:2:1.

Ligands and Receptor Preparation

The structures of the synthesized cadmium and cobalt complexes were drawn and standardized with MarvinsketchVersion 19.13, 2019, ChemAxon server at (http://www.chemaxon.com) and saved in SDF/3D format. *Trichophy tontonsuran*protein lanosterol 14α-demethylase was downloaded from Protein Data Bank at https://www.ncbi.nlm.nih.gov and saved in PDB format

Molecular Docking

Molecular docking of the ligands into the protein receptor/active site was done using AutodockVina with PyRx program. This was done by adjusting the docking parameters such as the grid box center: X= 170.754; Y= 146.896; Z= 118.8194 and grid box size: X= 55.1016; Y= 54.5187; and Z= 40.435Å. The standard ligand docked into the protein receptor site as obtained from the Protein Data Bank was also prepared and docked into the protein receptor as control and used as comparison to see the value of root-mean-square-deviation (RSMD). The docking software is preferred to predict result from experimental position with root-mean-square-deviation (RSMD) no more than 4.0Å. The docking results were then visualized using the PyMOL program. Observation of the interaction between the ligands and the receptor was done using the BIOvIADiscovery Studio Predictive Science Application. San Diego, Dassault Systems version 4.5.

The result of the scoring function otherwise known as binding affinity are chosen from the most negative values of the docking output. A low negative energy of interaction is indicative of a stable system and a very high likelihood of binding interaction between two molecules (Bissantz *et al.,* 2010; Pratama and Pratomo, 2017). Conclusions were drawn from the lowest negative binding affinity values than the counter and more amino acid residues bound by the same hydrogen bond as the control (Khazanov and Carlson, 2013; Agarwal *et al*., 2014;Chen *et al.,* 2016). The binding efficiency between a ligand complex and a receptor protein can be observed in the binding energy between the two and the ligand interaction with the receptor protein active site.

3. RESULT AND DISCUSSION

The melting points of the resulting metal complexes ranged range between 208 and 219 °C. The percentage yields were 37 % (1:1), 43 % (1:2), 58.6 % (1:1:1) and 61 % (1:2:1) with 202 – 204 °C, 204 – 208 °C, 214 – 216 °C and 217 – 219 °C melting points respectively for the resultant products of Cobalt(II)(Trimethoprim)$_2$ chloride complex. Cadmium (II) (trimethoprim) chloride and its mixed ligand gave white products with the percentage yields of 45 % (1:1), 59 % (1:2), 60 % (1:1:1) and 64 % (1:2:1) with the melting points of 200 – 202 °C, 204 – 206 °C, 228 -230 °C and 231 – 233 °C respectively (Table 1).

Table 1: Yield and melting point of metals complexes

Complex metals	Synthesis ratio	Yield (%)	Melting points (°C)
$CdLH_2OSO_4$	1:1	37.02	202 – 204
$CdL_2H_2OSO_4$	1:2	43 .01	204 – 208
$CdLH_2OSO_4$.SCN	1:1:1	58.60	214 – 216
$CdL_2H_2OSO_4$.SCN	1:2:1	61.00	217 – 219
$CoLClH_2O$	1:1	45.02	200 – 202
CoL_2ClH_2O	1:2	58.72	204 – 206
$CoLClH_2O$.SCN	1:1:1	59.74	228 -230
CoL_2ClH_2O.SCN	1:2:1	64.04	231 – 233

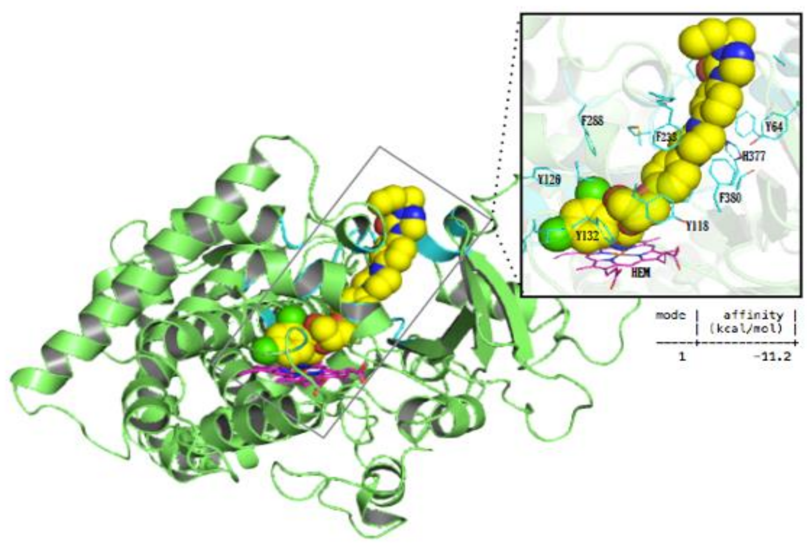
Table 2: Synthesis ratio and binding affinity of complex metals against lanosterol 14α-demethylase

Complex metals	Synthesis ratio	Binding Affinity kcal/mol
$CdLH_2OSO_4$	1:1	-8.0
$CdL_2H_2OSO_4$	1:2	-9.3
$CdLH_2OSO_4$.SCN	1:1:1	-7.5
$CdL_2H_2OSO_4$.SCN	1:2:1	-11.2
$CoLClH_2O$	1:1	-8.0
CoL_2ClH_2O	1:2	-9.3
$CoLClH_2O$.SCN	1:1:1	-7.5
CoL_2ClH_2O.SCN	1:2:1	-8.9
Co-Crystallized standard ligand (Control)		-11.2

Cadmium II complexes (Biswas *et al.,* 2014); cobalt II and cobalt III complexes have been reported to have antibacterial and antifungal properties. Similarly, trimethoprim and trimethoprim derivative are known to have antibiotic properties (Saadiyah *et al.,* 2010) and are used in the treatment of bacterial infections (Bean *et al.,* 2005) and have been reported to have significant antimicrobial activities (Roth *et al.,* 1962). The result of *in silico* study of the interaction between Cadmium or Cobalt trimethoprim thiocyanate complexes interactions with *Trichophyton* protein (lanosterol 14α-demethylase) are shown in table 1. The most negative binding affinity value is $CdL_2H_2OSO_4$.SCN against *Trichophyton* lanosterol 14α-demethylase with a binding value similar to the control at -11.2k.cal/mol with synthesis ratio 1:2:1 for cadmium, trimethoprim and thiocyanate respectively. Other complexes with close binding affinity are $CdL_2H_2OSO_4$ with binding affinity -9.3k.cal/mol and synthesis ratio 1:2 for cadmium and trimethoprim respectively, CoL_2ClH_2O with binding affinity -9.3k.cal/mol and synthesis ratio 1:2 for cobalt and trimethoprim respectively and CoL_2ClH_2O.SCN with binding affinity -8.9k.cal/mol with synthesis ratio 1:2:1 for cobalt, trimethoprim and thiocyanate respectively.

Figures 1 to 9 shows the best docking poses obtained for the interactions between the complex metals and the *C. albicans* protein lanosterol14α-demethylase and the amino acids residues performing important roles in the interactions. It was observed that the standard ligand docked into the protein receptor pocket obtained from protein data bank utilized 8 aromatic amino acid residues in its interactions with the *Trichophyton* protein as control. All of the amino acids are aromatic in nature. They are also either very hydrophobic or hydrophobic. The interaction between ligands and the hydrophobic side chains of proteins have been reported to contribute significantly to the binding free energy.

The hydrophobic amino acid residues mutually repel water and other polar groups which results in the net attraction of non-polar ligands thus contributing to a more negative binding free energy. Also, the presence of 6 apolar and aromatic rings of phenylalanine and tyrosine may have participated in stacking interactions with aromatic moieties of the ligand thus contributing to the most negative binding free energy. Twelve (12) amino acid residues were involved in the interaction between the ligand and the protein. All of which are either hydrophobic or very hydrophobic as shown in Figure 5.Studies have demonstrated that the hydrophobic interactions, quantified by the amount of hydrophobic surface buried upon ligand binding, is the structural parameter correlating best with binding free energy (Bissantz*et al.*, 2010, Perozzo *et al.*, 2004). Also, some of the amino acid residues are either aliphatic or aromatic in nature. Literatures have reported that perpendicular aliphatic-aromatic interactions provide a favourable contribution to binding free energy (Tsuzuki *et al.,* 2000).



Co-crystallized ligand

Fig. 1| **Co-crystallization of the fungal protein and the standard ligand**

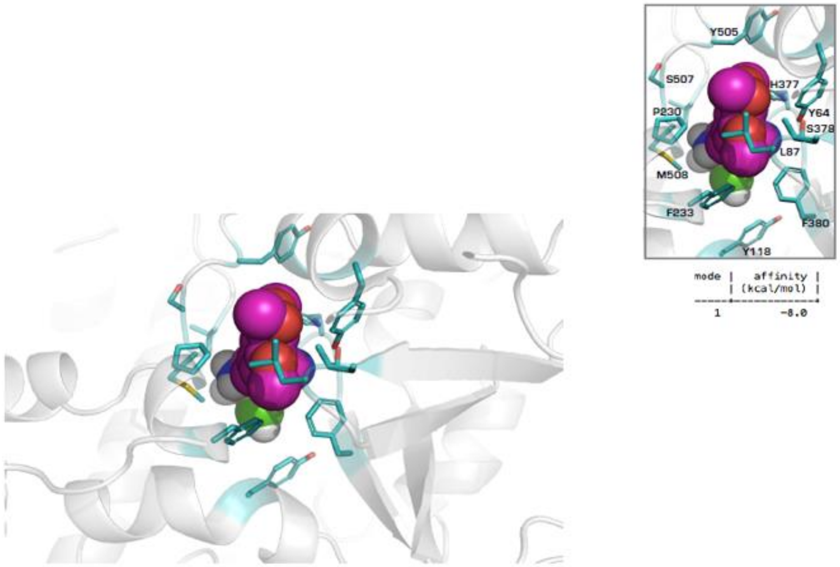


Fig. 2. Co-crystallized fungal protein Lanosterol 14α-demethylase and $CdLH_2OSO_4$ 1:1.

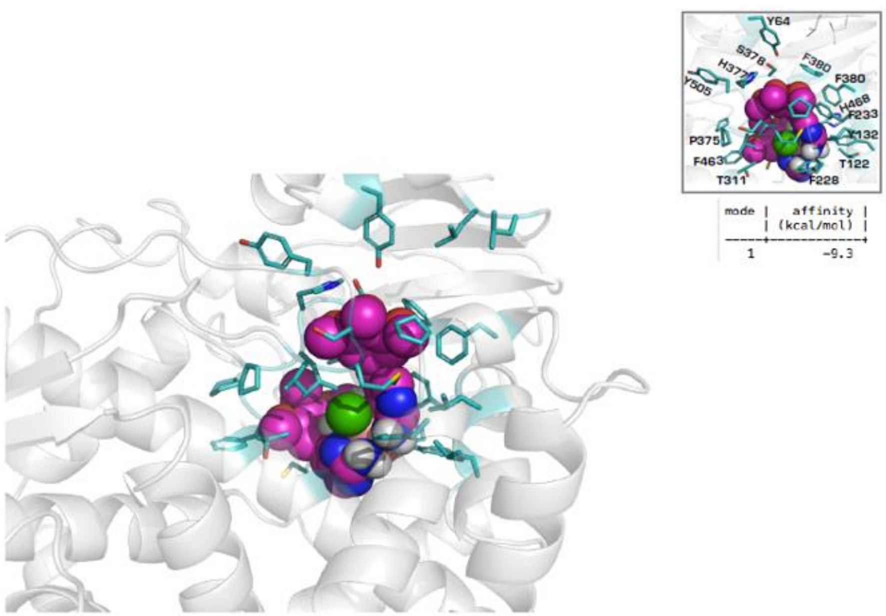


Fig. 3. Co-crystallized fungal protein Lanosterol 14α-demethylase and $CdL2H_2OSO_4$ 1:2

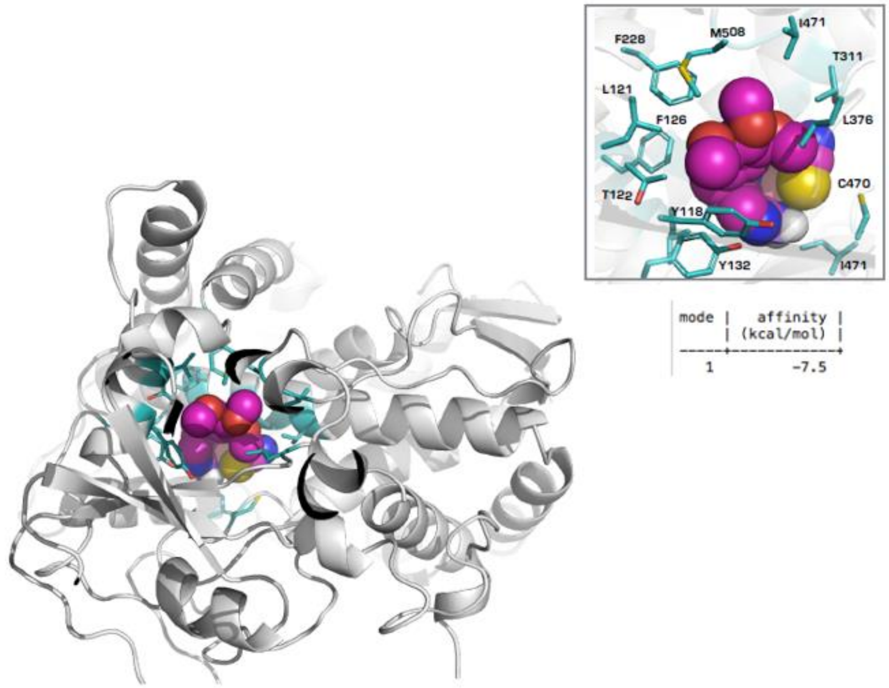


Fig. 4. Co-crystalized fungal protein Lanosterol 14α-demethylase and CdLH$_2$OSO$_4$.SCN.

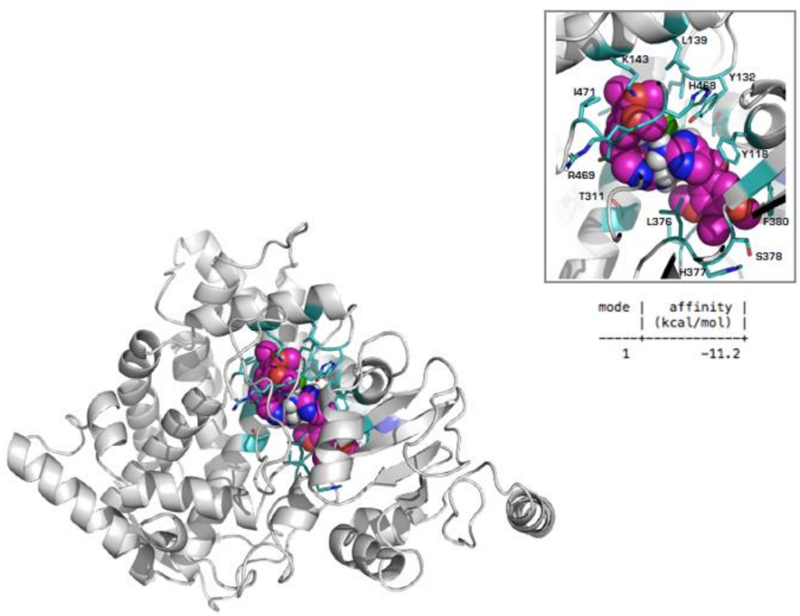
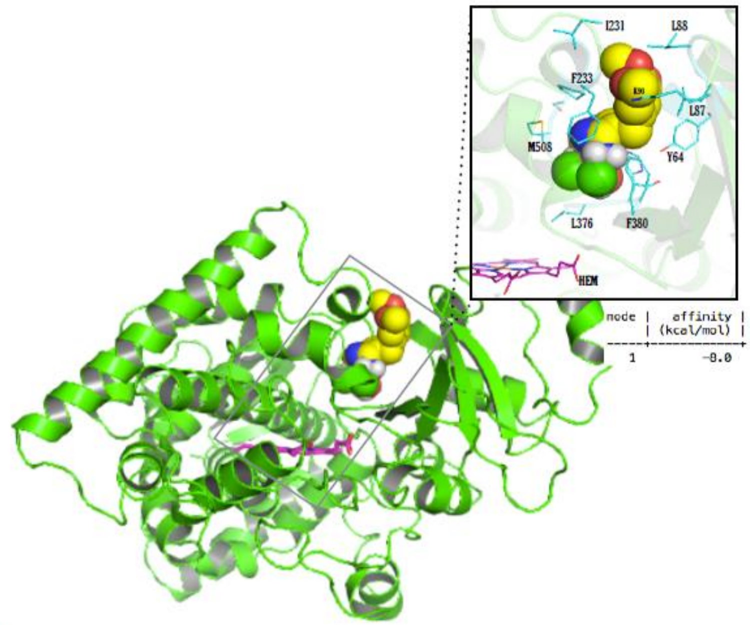
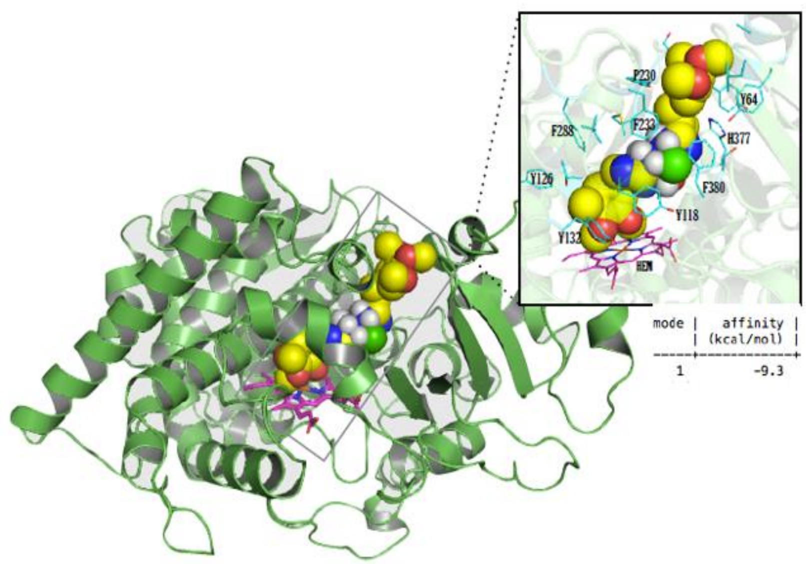


Fig. 5. Co-crystalized fungal protein Lanosterol 14α-demethylase and CdL2H$_2$OSO$_4$.SCN.



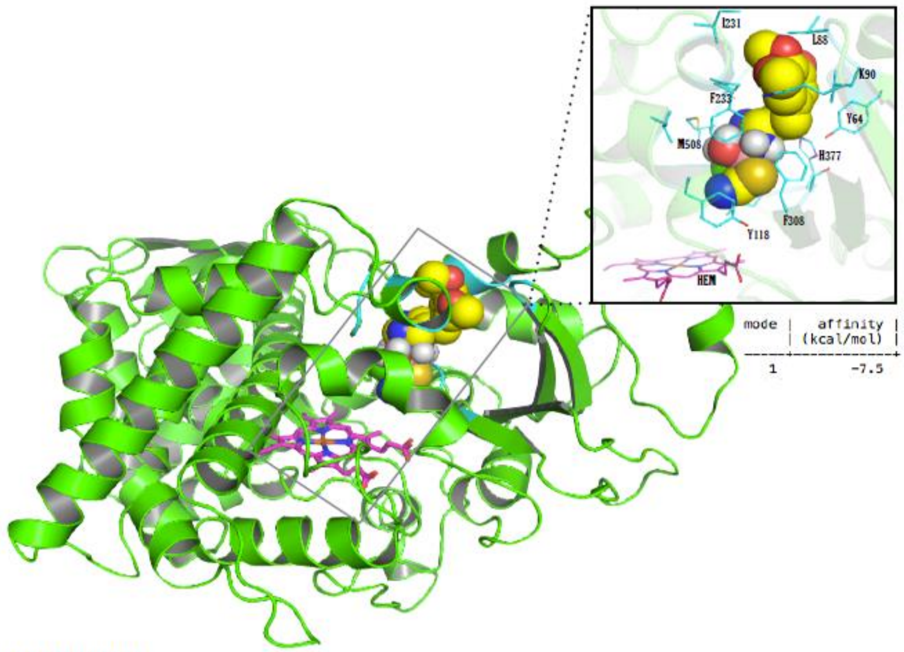
CoLCLH20

Fig. 6. Co-crystalized fungal protein Lanosterol 14α-demethylase and CoLClH$_2$O



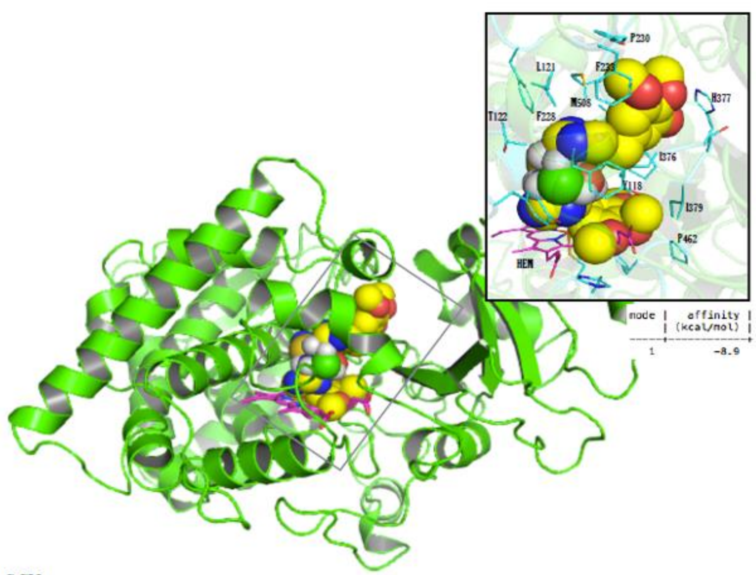
CoL2CLH20

Fig. 7. Co-crystalized fungal protein Lanosterol 14α-demethylase and CoL2ClH$_2$O



CoLCLH20SCN

Fig. 8| Co-crystalized fungal protein Lanosterol 14α-demethylase and CoLClH$_2$O.SCN



CoL2CLH20SCN

Fig. 9| Co-crystalized fungal protein Lanosterol 14α-demethylase and CoL2ClH$_2$O.SCN

It was also observed that all the complex metals cadmium and cobalt alike with 2 ratios of trimethoprim returned favourable values for binding free energy Figure 3 (-9.3 kcal/mol), Figure 5 (-11.2 kcal/mol), Figure 7 (-9.3 kcal/mol), and Figure 9 (-8.9 kcal/mol) while other complexes cadmium and cobalt alike with 1 ratio of trimethoprim gave docking output with higher binding free energies see Figure 2 (-8.0 kcal/mol), Figure 4 (-7.5 kcal/mol), Figure 6 (-8.0 kcal/mol) and Figure 8 (-7.5 kcal/mol). The presence of trimethoprim may have contributed to the stability of the interaction between the ligand and the protein (Muthiah and Robert, 1999).

4. CONCLUSION

This Study has reported that metal complex of purine and pyrimidine derivatives such as trimethoprim confer additional stability to coordination complexes through the presence of hydrogen bond between halogen ion and 2-amino group of the pyrimidine moiety. These synthesized complex metals showed promising anti-*Trichophyton* potentials.

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Data and materials availability: All data associated with this study are present in the paper.

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